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# Fortification of yoghurts with grape (*Vitis vinifera*) seed extracts

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ABSTRACT

The feasibility of full-fat and non-fat yoghurts fortification with grape seed extracts from two grape varieties, namely Moschofilero and Agiorgitiko, was examined. Epicatechin, total phenolics, antiradical activity, reducing power, viable *Lactobacilli* counts and pH of fortified and not fortified yoghurts were followed throughout products' shelf life. The fortification of yoghurts at 5–10 mg gallic acid equivalents/100 g of yoghurt did not affect yoghurt pH and *Lactobacilli* counts and – as perceived by the authors during tasting – did not cause major defects in consistency, colour and flavour compared to controls; differences in colour were detectable by colorimetry. Seed polyphenols were detected in supplemented yoghurts, in amounts proportional to those of seed extracts. Fortified yoghurts contained more polyphenols and exhibited higher antiradical and antioxidant activity than controls, even after 3–4 weeks of cold storage. The degradation of polyphenols and the decrement of yoghurts' antiradical and antioxidant activities followed first order kinetics, with full-fat yoghurts exhibiting higher deterioration rates and lower half-lives than the non-fat ones. It is concluded that, at the supplementation levels tested, the production of functional yogurts with grape-seed antioxidants is feasible, given that the supplementation is carried out in the fermented product and not in milk prior to fermentation.


## 1. Introduction

Yoghurt has gained a positive perception by consumers as a functional dairy product with health promoting ingredients (Allgeyer, Miller, & Lee, 2010). Consequently, yoghurt's consumption is increasing worldwide (Roberfroid, 2007), and it has more than doubled between the past 20 years in the US (Wallace & Giusti, 2008). Health benefits of yoghurt are correlated with the presence of living microorganisms like lactic acid bacteria, streptococci, bifidobacteria or their combinations, which originate from the starter cultures and are recognised as functional ingredients (Parvez, Malik, Ah Kang, & Kim, 2006; Puupponen-Pimia et al., 2002).

Yoghurt with added antioxidants from natural sources appears to be a convenient food format to satisfy consumer interest in original yoghurt nutrients, beneficial effects of starter cultures, and health benefits of added antioxidants. For this reason, several attempts to produce yoghurts fortified with natural antioxidant-rich extracts have been undertaken, including supplementation with polyphenol-rich wine extract (Howard, Nigdikar, Rajput-Williams, & Williams, 2000), *Hibiscus sabdariffa* extract (lwalokun & Shittu, 2007), pycnogenol from French marine bark extract (Ruggeri, Straniero, Pacifico, Aguzzi, & Virgili, 2008), quince scalding water (Trigueros, Pérez-Alvarez, Viuda-Martos, & Sendra, 2011), apple polyphenols (Sun-Waterhouse, Zhou, & Wadhwa, 2011), grape and grape callus extracts (Karaaslan, Ozden, Vardin, & Turkoglu, 2011), and tea infusions (Najgebauer-Lejko, Sady, Grega, & Walczycka, 2011).

Polyphenols are secondary metabolites synthesised by plants during normal development and/or as a response to environmental stress, wound or infection. They posses strong antioxidant activities acting as free radical scavengers, electron or hydrogen donors and strong metal chelators, preventing lipid peroxidation, DNA damage etc. Numerous studies have shown that polyphenols are important preventive agents against several degenerative diseases, clearly improving the status of different oxidative stress biomarkers (Pezzuto, 2008; Williamson & Manach, 2005), while there is now emerging evidence that their metabolites in the circulatory system

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exert modulatory effects in cells through selective actions on different components of the intracellular signalling cascades, vital for cellular functions such as growth, proliferation and apoptosis (Crozier, Jaganath, & Clifford, 2009).

Grape byproducts represent a rich source of phytochemicals, the recovery/reuse of which is of economic concern nowadays, as the disposal fees and fines for unauthorized discharges have increased considerably (Devesa-Rey et al., 2011). The annual production of grape byproducts – skins, seeds and stems – is estimated at 14.5 million tonnes, in Europe alone (Pinelo, Arnous, & Meyer, 2006). Grape seed is a winemaking by-product, with a characteristic flavanols profile and with significant total polyphenol content (Anastasiadi, Pratsinis, Kletsas, Skaltsounis, & Haroutounian, 2010; Arnous, Makris, & Kefalas, 2002; Guendez, Kallithraka, Makris, & Kefalas, 2005).

In the present study we evaluated the potential of using grape seed extracts from two Greek wine grape varieties for the production of antioxidant-rich full-fat and non-fat yoghurts. Taste and colour of control and supplemented yoghurts were recorded and compared. In addition pH, *Lactobacilli* viability, epicatechin, total polyphenol content, DPPH• scavenging activity and ferric ion reducing power (FRAP) of yoghurt samples were followed during 32 days of cold storage. To our knowledge this is the first study on the feasibility of employing grape seed extracts for the supplementation of full-fat and non-fat yoghurts with antioxidants.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Cinnamic acid, bis-(trimethylsilyl)-trifluoroacetamide (BSTFA), 3-(4-hydroxyphenyl)-1-propanol and oleanolic acid were obtained from Aldrich (Steinheim, Germany). Ascorbic acid, catechin, gallic acid, *p*-hydroxy-benzoic acid, *p*-hydroxy-phenylacetic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH•) stable radical, *p*-(dimethylamino)-cinnamaldehyde (DMACA), rutin and vanilin were from Sigma (Steinheim, Germany). Epicatechin, caffeic acid, protocatechuic acid, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®) were from Fluka (Steinheim, Germany). 2,4,6-Tri(2-pyridyl)-1,3,5-triazine (TPTZ), was from Alfa Aesar (Karlsruhe, Germany). Folin–Ciocalteu reagent, sodium nitrite, aluminium chloride hexahydrate and de Man–Rogosa–Sharpe (MRS) agar were from Merck (Darmstadt, Germany). All the solvents used were of HPLC grade and were purchased from Merck or Aldrich.

### 2.2. Grape seed extracts

Grape seeds were obtained from wineries located in Attica (central Greece), from two Greek grape varieties: "Moschofilero", red variety used for white wine production and "Agiorgitiko", red variety used for red wine production. Moschofilero seeds were collected immediately after processing of grapes, while those of Agiorgitiko were obtained from pomace disposed after remaining in contact with the fermenting must for 12 days. Following collection, seeds were transferred daily to the laboratory and stored at −40 °C. They were subsequently lyophilised, ground to a fine powder using a domestic blender and defatted with hexane by overnight stirring; after filtration, the solid residue was extracted twice more with hexane for 1 h. The defatting of grape seeds with hexane, a common solvent used for this purpose (Luque-Rodríguez, Luque de Castro, & Pérez-Juan, 2005; Yilmaz & Toledo, 2006), aimed to minimize subsequent extracts' oxidation. Antioxidant-rich extracts were obtained by the extraction of defatted seeds powder for 3 h with 50% (v/v) ethanol–water, at pH adjusted to 2 with citric acid. The extraction conditions had been optimized by factorial design and response surface methodology to obtain extracts with the higher radical scavenging capacity (Karvela, Makris, Kalogeropoulos, Karathanos, & Kefalas, 2009). It must be noted here that grape seed extracts have been approved as GRAS for use as a natural antioxidant and/or emulsifier in certain conventional foods (FDA, 2003).

### 2.3. Yoghurt fortification, bottling and storage

Agiorgitiko and Moschofilero seed extracts were used for the fortification of full-fat (FF, 4 g/100 g fat) and non-fat (NF, <0.5 g/100 g fat) yoghurts. Yoghurts were commercial products, purchased from local supermarkets on the day of fortification. According to their labels, the yoghurts were manufactured from cow's milk with the addition of mixed cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. According to the manufacturer, the yoghurts shelf lives were 26 and 33 days for FF and NF products, respectively. According to the manufacturer, the FF yoghurt contained by weight: cow's milk and concentrated skimmed cow's milk (96 g/100 g), milk cream (4 g/100 g), yoghurt culture (*L. bulgaricus* and *S. thermophilus*); its crude composition per 100 g was: proteins 4.5 g, fat 4.0 g, carbohydrates 6.5 g. The NF yoghurt contained by weight per 100 g: skimmed cow's milk and concentrated skimmed cow's milk (100 g), yoghurt culture (*L. bulgaricus* and *S. thermophilus*), while it's crude composition per 100 g was: proteins 5.0 g, fat 0 g, carbohydrates 7.5 g. Yoghurts total solids – calculated gravimetrically after samples' freeze drying – were 17.0 ± 0.003 g/100 g and 14.4 ± 0.004 g/100 g for FF and NF yoghurts, respectively. The yoghurts' pH were 4.45 ± 0.04 and 4.48 ± 0.03 for FF and NF samples, respectively; colour characteristics of the non supplemented yoghurts are provided in Table 2.

Attempts to produce fortified yoghurts were made by blending 40, 80 or 100 mg of dry seed extracts each time with 150 mL of homogenized milk, followed by inoculation with yoghurt culture and fermentation. Control yoghurt samples were prepared by inoculation of plain homogenized milk. When fortified and control samples were extracted (as described in 2.7) and analysed for total and simple polyphenols, DPPH• scavenging capacity and ferric reducing power, we did not observe differences between fortified and control samples at the supplementation levels tested. Thereafter, it was decided to carry the fortification directly in the plain yoghurts using the higher tested amount of seed extracts. For this purpose, 100 mg of dry seed extract was dissolved in 2 mL sterilised tap water, added in 150 g FF or NF yoghurt, and homogenised by manually mixing to avoid syneresis. Control – not fortified – FF and NF yoghurts were prepared by adding 2 mL of sterilised water to

**Table 1**
Total phenolics, radical scavenging capacity and antioxidant power of freeze dried grape seed extracts.

| Parameter | Agiorgitico seeds | Moschofilero seeds |
|---|---|---|
| Total phenolics (mg GAE/g dry extract) | 76.1 ± 6.7 | 151.6 ± 3.6 |
| Flavanols (mg CatE/g dry extract) | 35.5 ± 0.43 | 58.1 ± 0.68 |
| Flavones (mg RutE/g dry extract) | 1.89 ± 0.23 | 1.28 ± 0.10 |
| Proanthocyanidines (mg CyE/g dry extract) | 2.98 ± 0.13 | 6.10 ± 0.71 |
| Radical scavenging capacity (mg TE/g dry extract) | 58.9 ± 6.9 | 94.8 ± 5.8 |
| Ferric reducing antioxidant power (mg AAE/g dry extract) | 143.9 ± 13.7 | 202.5 ± 12.1 |

Results are the mean ± standard deviation of three determinations; GAE stands for gallic acid equivalents; CatE stands for catechin equivalents; RutE stands for rutin equivalents; CyE stands for cyanidin equivalents; TE stands for Trolox® equivalents; AAE stands for ascorbic acid equivalents.